

Attorney Docket No. 7593 CO1  
Customer No. 49459

### CLAIMS

Please consider the following amendments.

In the claims:

1. (Currently amended) A method of monitoring biofouling in a reverse osmosis membrane separation system including a reverse osmosis membrane capable of separating a feed stream into at least a permeate stream and a concentrate stream comprising the steps of:  
    providing a fluorogenic agent;  
    adding the fluorogenic agent to the feed stream;  
    providing a fluorometer to detect the fluorescent signal of the fluorogenic agent in ~~at least two of the feed stream~~ [[,]] ~~and the permeate stream and the concentrate stream;~~  
    reacting the fluorogenic agent with at least one microorganism within the reverse osmosis membrane separation system;  
    forming a reacted fluorogenic agent;  
    using the fluorometer to measure the fluorescent signal of at least one of the fluorogenic agent and the reacted fluorogenic agent in ~~at least two of the feed stream~~ [[,]] ~~the permeate stream,~~ and the concentrate stream; and  
    monitoring biofouling in the reverse osmosis membrane separation system based on the change in the signal of the fluorogenic agent, or the reacted fluorogenic agent or a combination of both signals measured.
2. (Original) The method of claim 1 wherein the membrane separation system is selected from the group consisting of a cross-flow reverse osmosis membrane separation system and a dead-end flow reverse osmosis membrane separation system.
3. (Original) The method of claim 1 wherein the fluorogenic agent is selected from the group consisting of acetic acid ester of pyrene 3,6,8-trisulfonic acid; carboxyfluorescein diacetate; 3-carboxyumbelliferyl  $\beta$ -D-galactopyranoside; 3-carboxyumbelliferyl  $\beta$ -D-glucuronide; 9H-(1,3-dichloro-9,0-dimethylacridine-2-one-7-yl), D-glucuronide; 9H-

Attorney Docket No. 7593 CO1  
Customer No. 49459

(1,3-dichloro-9,9-dimethylacridin-2-one-7-yl); resorufin  $\beta$ -D-galactopyranoside; fluorescein di- $\beta$ -D-galactopyranoside; fluorescein di- $\beta$ -D-glucuronide; resorufin  $\beta$ -D-glucuronide; fluorescein diphosphate; resazurin; resazurin, sodium salt; 4-methylumbelliferyl phosphate; 4-methylumbelliferyl  $\beta$ -D-glucuronide; pyranine phosphate; pyrene 3,6,8-trisulfonic acid 1-phosphate; and combinations thereof.

4. (Original) The method of claim 1 wherein the fluorogenic agent is selected from the group consisting of resazurin, 4-methylumbelliferyl phosphate, pyranine phosphate and combinations thereof.
5. (Original) The method of claim 1 wherein the fluorogenic agent is resazurin.
6. (Original) The method of claim 1 wherein the fluorogenic agent is added into the feed stream in an amount from about 0.5 ppb to 5 ppm.
7. (Original) The method of claim 1 wherein the fluorogenic agent is added into the feed stream in an amount from about 0.5 ppb to 5 ppm.
8. (Original) The method of claim 1 wherein the fluorogenic agent is added into the feed stream in an amount from about 5 ppb to about 500 ppb.
9. (Currently amended) The method of claim 1 wherein biofouling is monitored by determining a ratio of the fluorescent signal of the reacted fluorogenic agent to the fluorescent signal of the fluorogenic agent in ~~at least one of the concentrate stream and the permeate stream.~~
10. (Currently amended) The method of claim 9 further comprising the step of:  
determining the rate of change of the ratio of the fluorescent signal of the reacted fluorogenic agent to the fluorescent signal of the fluorogenic agent in ~~at least one of the concentrate stream and the permeate stream~~ to monitor biofouling.

Attorney Docket No. 7593 CO1  
Customer No. 49459

11. (Original) The method of claim 1 further comprising the step of:  
determining the optimal amount of biocontrol treatment based on the change in the signal of the fluorogenic agent, or the reacted fluorogenic agent, or a combination of both signals measured; and applying the optimal amount of biocontrol treatment to the membrane separation system.
12. (Original) The method of claim 11 wherein the biocontrol treatment is selected from the group consisting of biocides, biocontrol agents, biocontrol methods and combinations thereof.
13. (Original) The method of claim 12 wherein the biocides are selected from the group consisting of oxidizing biocides, non-oxidizing biocides and combinations thereof.
14. (Original) The method of claim 12 wherein the biocontrol agents are selected from the group consisting of bio-dispersants, bio-detergents, chaotropic agents, surfactants, chelating agents, enzymatic cleaners and combinations thereof.
15. (Original) The method of claim 12 wherein the biocontrol methods are selected from the group consisting of ultrasound, electric fields and air backwashes.
16. (Original) The method of claim 1 wherein the microorganisms are selected from the group consisting of planktonic microorganisms, sessile microorganisms and combinations thereof.
17. (Original) The method of claim 1 further comprising the addition of an inert fluorescent tracer to the feed stream to monitor biofouling in the membrane separation system based on the change in the signal of the fluorogenic agent or the reacted fluorogenic agent relative to the signal of the inert fluorescent tracer.